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## HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

# Estrogen Receptor Gene Polymorphisms and the Genetics of Osteoporosis: A HuGE Review

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Osteoporosis (OMIM166710) is a common skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue with increased susceptibility to fracture. Osteoporosis has a complex etiology and is considered a multifactorial polygenic disease in which genetic determinants are modulated by hormonal, environmental, and nutritional factors. Estrogens are known to play an important role in regulating bone homeostasis and preventing postmenopausal bone loss. They act through binding to two different estrogen receptors (ERs), ERα (OMIM133430) and ERβ (OMIM601663), which are members of the nuclear receptor superfamily of ligand-activated transcription factors. Different polymorphisms have been described in both the *ER*α and *ER*β genes. Although a large number of association studies have been performed, the individual contribution of these polymorphisms to the pathogenesis of osteoporosis remains to be universally confirmed. Moreover, an important aim in future work will be to define their functional molecular consequences and their interaction with the environment in the causation of the osteoporotic phenotype. A further promising application of these polymorphisms comes from their pharmacogenomic implications, with the possibility of providing better guidance for therapeutic regimens, such as estrogen replacement therapy and selective ER modulators. At the moment, no recommendations for population-based screening can be made.

epidemiology; estrogen receptor alpha; estrogen receptor beta; genetics; genome, human; osteoporosis; polymorphism, genetic

Abbreviations: BMD, bone mineral density; ER, estrogen receptor; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; VDR, vitamin D receptor; VNTR, variable number of tandem repeats.

***Editor's note:*** *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

### GENE(S)

Estrogens exert beneficial effects on the development and maintenance of the skeleton (1–3). These include control of growth plate maturation and closure during longitudinal growth, regulation of cortical and cancellous bone metabolism, acquisition of peak bone mass, and inhibition of bone loss. Recent reports from population-based studies clearly indicate that estrogens are necessary for regulation of skeletal homeostasis not only in women but also in men (4–6). The skeletal effects of estrogen are mediated by its binding to specific estrogen receptors (ERs) localized at the cytosolic and nuclear level. These receptors belong to the nuclear receptor hormone superfamily and are ligand-inducible transcription factors.

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Two functional ERs, namely ERα and ERβ, which are encoded by different genes, have been described so far. The human *ER*α gene (also named *ESR1*) is located on chromosome 6q25. It comprises eight exons separated by seven intronic regions and spans more than 140 kilobases (7, 8). The *ER*β gene (*ESR2*) is located on chromosome 14q23–24.1 and is composed of eight exons spanning approximately 40 kilobases (9, 10). *ER*β is smaller than *ER*α but possesses a similar structure and considerable homology in the DNA-binding and ligand-binding domain (11). Both ER isoforms are expressed in osteoblasts, osteoclasts, and bone marrow stromal cells (1, 12) and co-localize in adult bone. There is also evidence of age- and gender-specific expression of ERβ protein (13). Some studies have suggested that ERβ is more ubiquitously expressed with higher levels than ERα in trabecular bone (14, 15). In contrast, ERα predominates in cortical bone (16).

ERα appears to be the major receptor mediating estrogen action in bone, and it has a prominent effect on the regulation of bone turnover and the maintenance of bone mass. Although the role of the recently discovered ERβ in bone remains unknown, there is a range of molecular evidence from in vitro studies and animal models that points toward a function distinct from that of ERα. Mice with null mutations in the *ER*α and *ER*β genes show distinct skeletal phenotypes. In particular, ERβ may have opposing effects than ERα in mediating estrogenic action on longitudinal bone growth and bone size, with only a partial and redundant effect on trabecular bone mineral density (BMD) (17–21). Functional ERs are usually homodimers, but ERα/ERβ heterodimers have been also described (22). Importantly, ERβ can inhibit transcriptional activation of ERα through formation of these heterodimers, as shown by different in vitro and in vivo experimental studies (23–25). However, in the absence of ERα, ERβ is able to replace some of its activity (25).

## GENE VARIANTS

### Estrogen receptor α

Genetic screening of the *ER*α gene locus has revealed the existence of several polymorphic sites (26–31). An updated view of the *ER*α gene structure with its polymorphic regions is shown in figure 1. The most widely studied are the *Pvu*II (T397C) and *Xba*I (C351G) restriction fragment length polymorphisms (RFLPs) in intron I and the $(TA)_n$ variable number of tandem repeats (VNTR) within the promoter region of the gene. In different studies, these polymorphisms have been associated with several pathologic conditions such as breast and prostate cancer, osteoporosis, Alzheimer's disease, and cardiovascular diseases (31–37). However, results are still conflicting and the molecular mechanisms by which these polymorphisms influence receptor activity are as yet unclear. *Pvu*II and *Xba*I RFLPs lie in an intronic and apparently nonfunctional area of the gene and, as would be expected for two polymorphisms separated by 50 base pairs, are in strong linkage disequilibrium. Alleles *P* and *X* (absence of restriction sites), as well as alleles *p* and *x* (presence of restriction sites), are strongly associated with each other. However, haplotype *pX* was not observed in the majority of studies, whereas haplotype *Px* was detected, albeit at a low frequency; this indicates that the disequilibrium is not complete and that either recombination or multiple mutations have occurred between or at these two polymorphic sites.

Recently, Herrington et al. (38) noted that the T→C transition associated with loss of the *Pvu*II site (*P* allele) results in a potential binding site for *myb* transcription factors that, in the presence of B-*myb*, is capable of augmenting in vitro transcription of a downstream reporter construct 10-fold. Thus, in some settings, the presence of the *P* allele might amplify *ER*α transcription. An alternative explanation is that the two polymorphisms in intron I may be in linkage disequilibrium with causal polymorphisms elsewhere in the *ER*α gene or, less likely, in an adjacent gene. In this regard, it has been well established that intron I polymorphisms are in linkage disequilibrium with the upstream TA repeat polymorphism in the promoter region of the *ER*α gene (39). Previous studies have shown that VNTR polymorphisms in the proximity of some gene promoters can have a significant influence on transcriptional regulation (40). It is conceivable that the number of TA repeats could be important for *ER*α gene transcription. To date, at least three different promoters have been identified in the *ER*α gene, and several sites of transcription initiation from these promoters have been suggested (41–49). Because of its position between the promoter A and B regions, its has been speculated that allelic variation due to different TA repeat lengths might have physiologic relevance by affecting promoter usage (39). Moreover, a novel regulatory element resembling a steroid response element has recently been identified in the 5′-flanking region of the human *ER*α gene, approximately 220 bases downstream from the $(TA)_n$ VNTR (50). It has been demonstrated that this sequence acts as a strong enhancer element in several cell lines (50). There are well-established population differences in the TA repeat length, as well as in the allelic frequency of *Pvu*II and *Xba*I RFLPs. The distribution of the TA repeat alleles differs slightly between populations of European and Asian ancestry, with major peaks at 14 and 15 repeats, respectively (figure 2). Even though slight ethnic differences in genotype distributions of the *Pvu*II and *Xba*I RFLPs have been described, important variations have been observed concerning the *Pvu*II-*Xba*I haplotypes (table 1). Asian populations showed an increased frequency of the *Px* haplotype and a reduced frequency of the *PX* haplotype with respect to Caucasian populations of European ancestry, while in an African population haplotype *px* was present at a lower frequency (51).

Other polymorphisms have recently been described in the *ER*α gene. These include a T262C single nucleotide polymorphism 29 nucleotides downstream from the putative start codon, a C→G single nucleotide polymorphism in codon 325 in exon 4, and a G2014A single nucleotide polymorphism in exon 8 (52–54). All three of these single nucleotide polymorphisms are silent, since they do not cause any amino acid change. In one study (52), the G2014A single nucleotide polymorphism in exon 8 was shown to be in linkage disequilibrium with the *Pvu*II polymorphism in intron I.

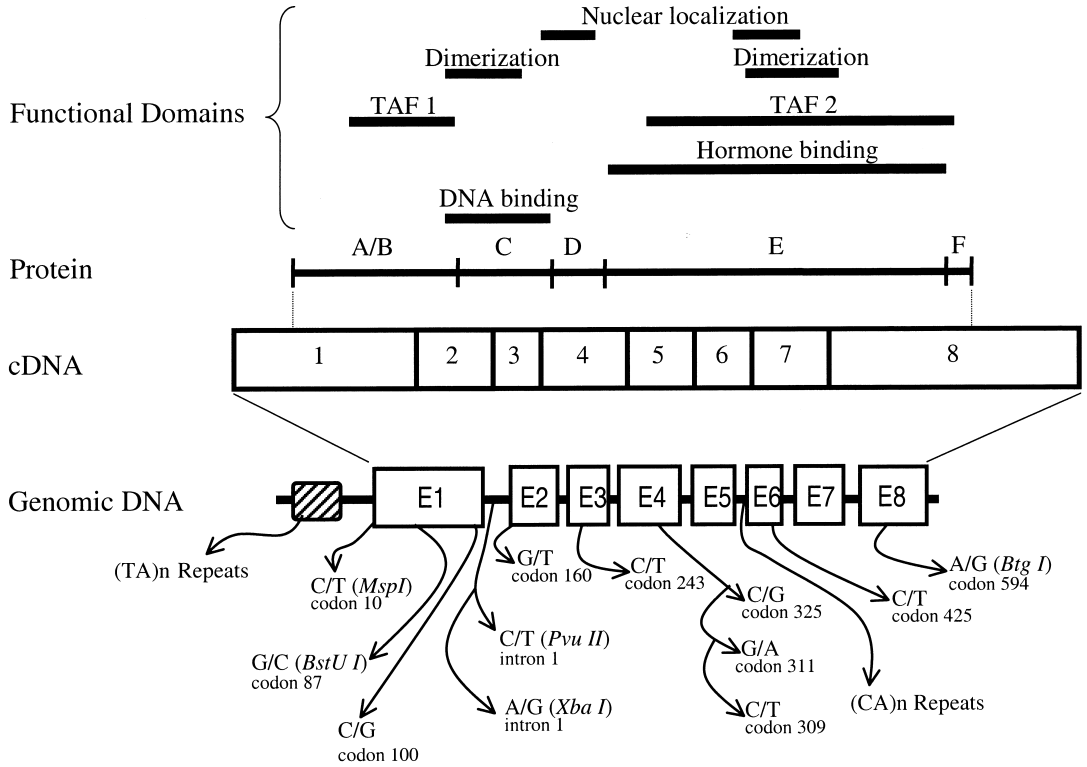


**FIGURE 1.** Structure of, functional domains of, and described polymorphisms in the human estrogen receptor α gene. Coding exons (E) are indicated with boxes. TAF, transcriptional activating function.

### Estrogen receptor β

In 1998, Tsukamoto et al. (55) firstly characterized a highly polymorphic (CA) dinucleotide repeat in intron 5 of the human *ER*β gene in a Japanese population. Subsequently, systematic mutation screening detected five different sequence variants, including two mutations and three polymorphisms (56). The first was a silent T1421C transition in exon 7; the second was a silent G1082A transition within the ligand-binding domain in exon 5; and the third was a A1730G single nucleotide polymorphism in the 3′-untranslated region of exon 8. The functional importance of these polymorphisms has not been clarified, and differences in the distribution of genotypes between Caucasian and Asian populations have been described. More recently, five novel polymorphisms were identified in an African population (57). Three of them (C143T in exon 1, A566T in exon 2, and T1100G in exon 5) are silent polymorphisms, while the other two are expected to change the amino acid sequence of ERβ. These include an A105G single nucleotide polymorphism in exon 1 corresponding to an isoleucine-to-valine substitution at amino acid position 3 and a T1057G single nucleotide polymorphism in exon 5 determining a valine-to-glycine substitution at position 320, in helix 4 of the ligand-binding domain. Helix 4 does not participate directly in ligand binding, but it interacts with coactivators. Importantly, in functional in vitro analysis, the presence of the valine in position 320 showed significantly decreased maximal transcriptional activity (57). The characterized polymorphic variants in the *ER*β gene are summarized in figure 3.

## DISEASE

Osteoporosis is the most prevalent metabolic bone disorder among developed countries. It is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to nontraumatic fracture (58). Osteoporosis is now considered a major and growing health-care problem throughout the world. It has been estimated that in the United States, at least 90 percent of all hip and spine fractures among elderly White women and more than 70 percent of those among elderly White men may be attributed to osteoporosis (59). The lifetime risk of fracture in women aged 50 years is approximately 16 percent for hip fractures, 15 percent for wrist fractures, and 32 percent for vertebral fractures (60). Approximately 50 percent of all women will have osteoporosis by the age of 80 years. Conversely, a White man aged 50 years has approximately a 6 percent risk of hip fracture and a 16–25 percent risk of any osteoporotic fracture in his remaining life (61). It has been estimated that costs related to hip fracture will double during the next 25 years (62). Because of the increasing life expectancy of the population, the number of people with osteoporosis will be augmented drastically during the coming years, with huge health implications. Therefore, prevention

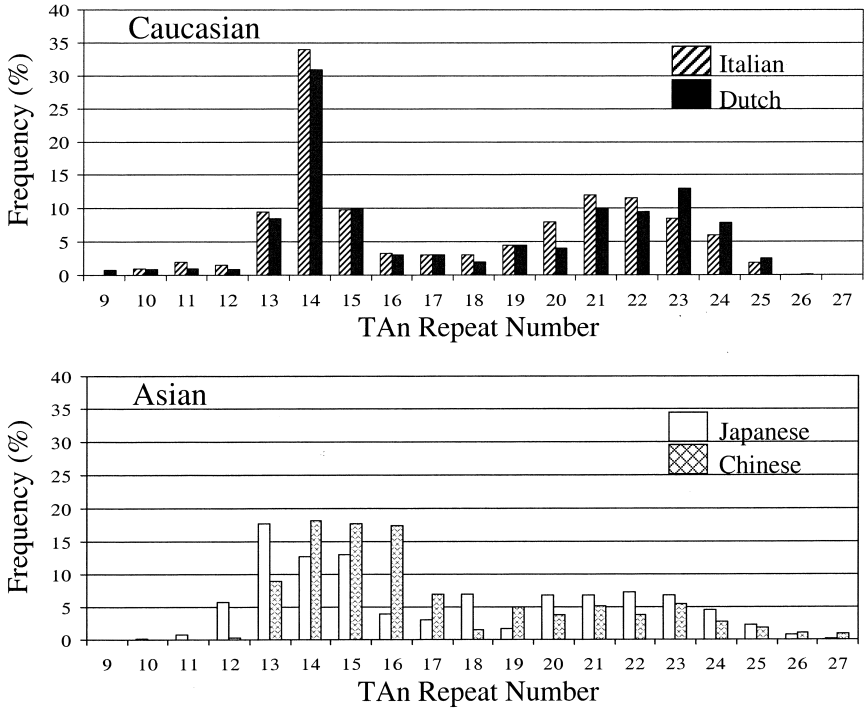


**FIGURE 2.** Distribution of $(TA)_n$ repeat alleles in the human estrogen receptor α gene in different ethnic groups. Top: Major studies in Italian (39) and Dutch (51) Caucasian populations. Bottom: Major studies in Japanese (75) and Chinese (118) Asian populations.

and treatment of osteoporosis is of major importance for health organizations in all countries.

Osteoporosis affects mainly postmenopausal women, but also men. Osteoporotic fractures, which represent the most relevant clinical aspect of the disease, usually occur in the distal forearm, thoracic and lumbar vertebrae, and proximal femur. Their incidence increases with age and is higher in Whites than in Blacks. Fractures of the hip incur the largest direct cost for health services and mainly occur in the elderly, giving rise to substantial morbidity and mortality. Osteoporotic fractures of the vertebrae and forearm are of less economic significance but also give rise to significant morbidity. Quality of life becomes progressively impaired as the number and severity of vertebral fractures increases. Moreover, future risk of osteoporotic fractures is greatly increased in patients with one or more vertebral fractures.

The major determinant of bone strength and osteoporotic fracture risk is BMD, as assessed by dual photon absorptiometry or dual energy x-ray absorptiometry (63–66). In women, according to World Health Organization criteria,

**TABLE 1. Frequency of the combined *Pvu*II-*Xba*I haplotypes of the human estrogen receptor α gene in different ethnic groups**

| Ethnic group | Place of study | No. of subjects | *Pvu*II and *Xba*I haplotypes* | | | | Study (reference no.) |
|---|---|---|---|---|---|---|---|
| | | | *px* | *PX* | *Px* | *pX* | |
| Asians | Japan | 238 | 54.5 | 18.7 | 26.5 | 0.3 | Kobayashi et al. (74) |
| Asians | Japan | 2,238 | 59.4 | 18.3 | 22.3 | 0 | Yamada et al. (84) |
| Asians | Korea | 598 | 57.7 | 18.5 | 21.5 | 2.3 | Han et al. (77) |
| Caucasians | Russia | 344 | 61.5 | 28.6 | 9.9 | 0 | Sapir-Koren et al. (106) |
| Caucasians | Denmark | 454 | 53.0 | 33.7 | 13.3 | 0 | Bagger et al. (95) |
| Caucasians | The Netherlands | 1,100 | 53.0 | 36.1 | 10.9 | 0 | van Meurs et al. (51) |
| Caucasians | United Kingdom | 206 | 56.1 | 33.5 | 9.2 | 1.2 | Albagha et al. (101) |
| Caucasians | Italy | 610 | 52.1 | 40.9 | 5.7 | 1.3 | Becherini et al. (39) |
| Caucasians | Canada | 662 | 54.9 | 35.6 | 9.5 | 0 | Patel et al. (134) |
| African Americans | New Jersey, United States | 19 | 36.8 | 50.0 | 13.6 | 0 | van Meurs et al. (51) |

* Alleles *P* and *X*, absence of restriction sites; alleles *p* and *x*, presence of restriction sites.

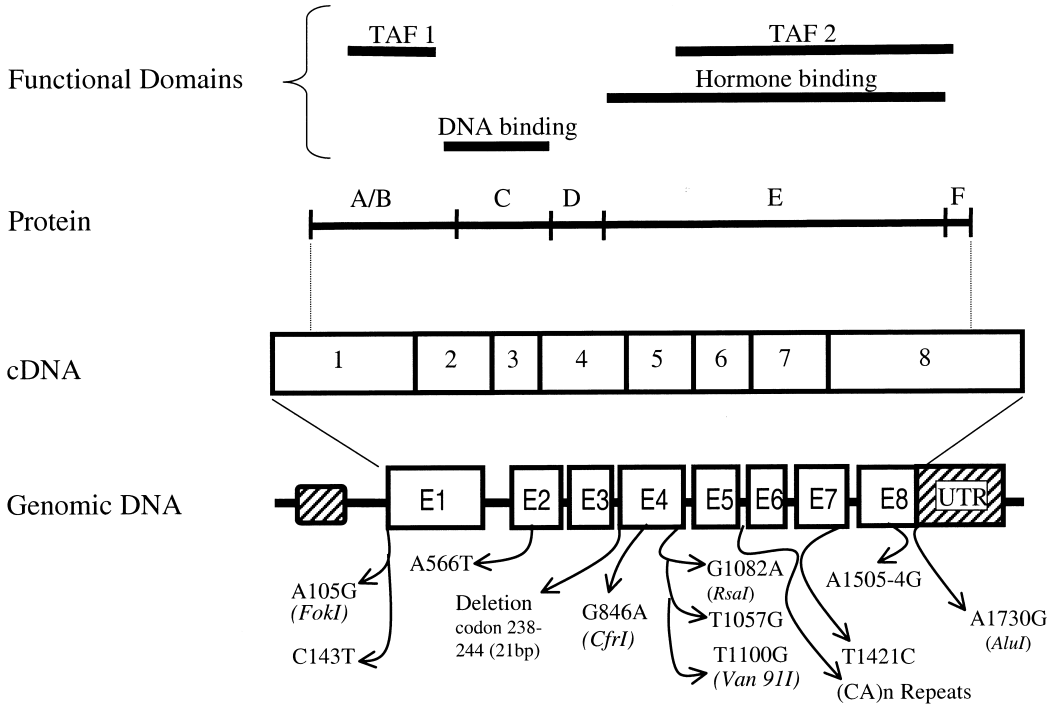


**FIGURE 3.** Structure of, functional domains of, and described polymorphisms in the human estrogen receptor β gene. Coding exons (E) are indicated with boxes. TAF, transcriptional activating function; UTR, untranslated region.

osteoporosis is defined to exist when BMD values fall more than 2.5 standard deviations below the young adult reference mean (63). Many studies have indicated that the risk of fragility fractures increases progressively as BMD declines and that there is a close relation between the prevalence of osteoporosis, as assessed by World Health Organization criteria, and the incidence of fractures (60, 64–66). It has been estimated that the risk of new vertebral fractures increases by a factor of 2.0–2.4 for each standard deviation decrease in BMD, irrespective of the site of measurement (67). Bone strength does not depend only on BMD; bone size and bone quality are other important components. A longer hip axis length is also associated with increased hip fracture risk independently of BMD (68). Several other factors contribute to the risk of osteoporotic fractures, including muscle weakness, impaired vision, and cognitive impairment (69). Trauma, such as a fall, is an important factor as well. Any bone will fracture if subjected to excessive force; however, the lower is BMD the higher is the risk of fracture. A bone mass below average for age can be considered a consequence of inadequate accumulation of bone in young adult life (low peak bone mass) or excessive rates of bone loss or both of these conditions.

Osteoporosis has a complex multifactorial pathogenesis. Although there are several environmental influences on BMD, such as diet (calcium intake and alcohol consumption) and lifestyle factors (smoking and physical exercise), a genetic contribution to the pathogenesis of osteoporosis, accounting for 50–80 percent of the interindividual variability in bone mass, has been recognized (70, 71). There are many candidate genes implicated in the determination of BMD and in the pathogenesis of osteoporosis, spanning from those encoding for cytokines through those encoding for calciotropic hormones and their receptors to the ones encoding for collagenic and noncollagenic proteins of bone. As mediators of estrogen action, the genes encoding ERα and ERβ have been considered important candidates for the determination of osteoporotic risk. The clinical observation of an osteoporotic phenotype in a man with a disruptive mutation of the *ERα* gene (72), as well as the report of decreased BMD values in mice lacking functional ERα (17–20), support the hypothesis of *ERα* as a likely candidate gene for osteoporosis. It is possible that common allelic variants of the *ERα* gene causing differential responsiveness to estrogen exist in the general population and that high estrogen levels can initially overcome the resistance, resulting in a normal phenotype. This compensatory balance could be altered later on by aging or by a condition such as menopause, leading to clinical disorders such as osteoporosis. Moreover, results from a recent genome-wide BMD linkage analysis in extended pedigrees from the Framingham Study provided evidence for a possible linkage of a region on chromosome 8 containing the *ERβ* gene with lumbar BMD (73).

## ASSOCIATIONS

We appraised the results of studies of the above polymorphisms and osteoporosis. These studies were identified through a Medline (PubMed) search of articles published between January 1990 and June 2004. We combined searches for the keywords "estrogen receptor," "polymor-

phism," "BMD," and "osteoporosis" to identify relevant studies. We further searched the references of any identified paper to locate additional studies. Results are summarized in Web tables 1, 2, and 3, which are available on the *Journal*'s website (http://www.aje.oupjournals.org/).

### Estrogen receptor α

Both intron I polymorphisms (*Pvu*II and *Xba*I) and the VNTR TA polymorphism upstream from the *ERα* gene were first associated with BMD variation in the Japanese population (74, 75). Japanese postmenopausal women who were homozygous for the absence of the *Xba*I restriction site and for the presence of the *Pvu*II restriction site (*Px* homozygous haplotype), as well as those with a number of 12 TA repeats, were at increased osteoporotic risk, showing the lowest BMD values. Subsequent studies searching for an association between intron I RFLPs and BMD in other Asian populations yielded conflicting results (76–92). No significant associations were observed in studies of Korean (76, 77) or Chinese (78) populations. Other confirmatory studies generally showed an association between the *PP* and/or *xx* genotypes and reduced BMD (79, 80, 85, 88–90, 92), but opposite associations were also described (81, 82, 86, 87, 91). The largest study performed to date reported an association between the *PX* haplotype and reduced BMD in elderly Japanese women (84). Moreover, a single study examined the effect of these polymorphisms in 401 Chinese nuclear families, showing minor but significant effects on BMD variation by linkage tests (83).

Similar observations among White Caucasian subjects showed conflicting results. Studies in Belgian (93, 94), Danish (95), Italian (96), and Australian (97) populations failed to find any significant association between the *Xba*I and/or *Pvu*II genotypes and BMD or bone accrual (98). Conversely, the *pp* genotype was associated with the lowest lumbar and total body BMD values in a group of US pre- and perimenopausal women from the Michigan Bone Health Study (99). A subsequent longitudinal evaluation in the same cohort of women demonstrated increased rates of BMD change in subjects with either the *xx* or the *pp* genotype (100). The latter observation is in keeping with a recent large-scale analysis carried out among elderly subjects from the Rotterdam Study (51); the investigators described an association of the "*px* haplotype" with reduced BMD and increased fracture risk, with evidence for an allele dose effect (for allele copy, odds ratio = 2.2). On the other hand, a study of Scottish postmenopausal women showed lower BMD values in subjects with the *Px* haplotype than in those with the *PX* and *px* haplotypes (101). The same haplotype (*Px*) appeared to be associated with low BMD values in a pedigree study in the Chuvaska population in Russia (102).

Studies on bone turnover and rates of bone loss according to different *Pvu*II and/or *Xba*I RFLPs also produced conflicting results (74, 77, 79, 81, 93–95, 99, 103, 104).

Data on the relation between the *ERα* polymorphism and BMD in men are limited but still discordant. Positive associations were reported in four studies of elderly men from Thailand (105), Israel (106), Finland (107), and China (108). Similar studies in British (109), Japanese (84), Korean (110), Dutch (51), and North American (111) populations did not detect any significant association. A single study examined the effect of these polymorphisms in adolescent boys, showing evidence for an association between the *xx* or *pp* genotype and increased bone density (112).

In summary, the association between intronic *Pvu*II-*Xba*I RFLPs and osteoporotic risk remains controversial. A recent meta-analysis combining the results of heterogeneous studies published through November 2001 evidenced a protective role of the *XX* genotype in BMD and fracture risk, with no apparent effect of the *Pvu*II polymorphism (113). Various suggestions have been made to account for these discordant findings, including genotyping error, ethnic or environmental differences among populations, differences in age and menopausal status, the inadequate sample size of many studies, treatments (i.e., estrogen use), study design (population- versus hospital-based case-control studies, cohort studies), and the health-based selection bias, with its tendency to exclude osteoporotic subjects. Even though minor ethnic variations exist in the distribution of the *Xba*I and *Pvu*II genotypes (114), the degree of disequilibrium may vary among different ethnic groups, since the frequency of the *Px* haplotype is increased approximately twofold in subjects of Asian ancestry with respect to Caucasian populations (51). The latter observation may explain, at least in part, the observed population specificity in the genotypes predicting low and high BMD. In fact, in the majority of studies performed in populations of Asian origin, the *PP* and/or *xx* genotypes were associated with the lowest BMD values, while in Caucasian populations, the *pp* and/or *xx* genotypes were shown to confer increased risk of osteoporosis. An interesting additional hypothesis has been recently proposed by Khosla et al. (111), who suggested that the *pp* or *xx* genotype may be relatively estrogen-insensitive and that subjects with the *P* or *X* allele may benefit more from the protective effects of estrogen on bone than subjects with the *p* or *x* allele. Thus, positive or negative associations may be dependent on circulating estrogen levels. A potential confounder in such studies may also be recognized in gene-gene interactions (115).

The original association between the variable number of TA repeats and BMD that was described in the Japanese population (74, 75) was confirmed in most of the studies that followed (39, 51, 101, 116, 117). In two large-scale studies performed in Italian and Dutch populations, a statistically significant correlation between the TA repeat length and fracture risk was also observed. Women with a low number of repeats showed lower BMD and a higher incidence of vertebral fractures in comparison with women with a high repeat genotype, equivalent to a two- to threefold increase in vertebral fracture risk (39, 51). A similar association between a low TA repeat number and decreased BMD was observed in pre- and perimenopausal women from the Michigan Bone Health Study but not in young children from the Iowa Bone Development Study (98, 117). By contrast, the opposite association (lowest BMD in women with a high number of repeats) was reported in a sample of Chinese women from Taiwan (118). Finally, no overall association between TA repeat number and BMD was evident in a study on Scottish postmenopausal women (101).

Other polymorphisms within the *ERα* gene (i.e., the exon 8 G2014A and exon 4 codon 325 C/G single nucleotide polymorphisms) have been associated with BMD and osteoporosis in single studies (53, 54, 119, 120).

### Estrogen receptor β

An association between the dinucleotide $(CA)_n$ repeat polymorphism and BMD was originally described in the Japanese population (121). Japanese postmenopausal women possessing at least one allele with 26 CA repeats had significantly higher BMD at the lumbar spine compared with those not possessing the 26 CA allele. The same polymorphism was also associated with levels of androgen and sex hormone-binding globulin in premenopausal women of European descent (122), with possible implications for bone turnover. Two other studies recently confirmed the association between the CA repeat polymorphism and BMD but with slightly different results (123, 124). In a cohort of Chinese women from Hong Kong, the 20 CA repeat allele was associated with high BMD in premenopausal women but not in postmenopausal women (123). Scariano et al. (124), in a study of postmenopausal US women from New Mexico, observed a higher BMD in subjects with a low number of CA repeats (CA <25) as compared with those having longer alleles. In contrast, no significant associations between the CA repeat length and longitudinal change in BMD or markers of bone metabolism were detected in this population, indicating that this polymorphism is associated with the attainment of peak bone mass rather than bone loss (124). Importantly, a recent large-scale analysis of the CA repeat polymorphism within the Framingham Study offspring cohort confirmed the significant association between the number of CA repeats and femoral BMD but not spinal BMD (125). The lowest BMD values were observed in subjects who were homozygous for a high number of repeats (CA ≥23). In the same cohort, two intronic single nucleotide polymorphisms (rs1256031 and rs1256059) exhibited an association with femoral BMD in men but not in women. When the three significant polymorphisms were analyzed together, haplotype C-23CA-T, with a frequency of 0.09, was significantly associated with lower femoral BMD in both sexes. Two other single nucleotide polymorphisms (rs1256034 and rs944460) with minor allele frequencies (<4 percent) gave no evidence of association (125). Arko et al. (126) examined the *Rsa*I polymorphism in exon 5 of the *ERβ* gene in a small sample of postmenopausal Slovenian women, reporting no association. In a preliminary study in 300 postmenopausal Italian women, Becherini et al. (127) reported no significant effect of the G1082C single nucleotide polymorphism on BMD or vertebral fractures.

## INTERACTIONS

Since bone mass and osteoporotic fracture risk are multifactorial traits, there are several possible interactions between *ER* polymorphisms and effect modifiers such as age, gender, diet, habits, and other environmental factors. Moreover, as with other complex traits, BMD values are likely to be determined by several genes that act collectively, and allelic variants at different genes may have either additive or contrasting effects on bone.

### Gene-gene interactions

Investigators from several studies have proposed the existence of a significant gene-gene interaction effect between intron I *Pvu*II-*Xba*I RFLPs in *ERα* and vitamin D receptor (*VDR*) gene polymorphisms in the determination of BMD or calcaneal ultrasonic parameters in pre- and postmenopausal women (82, 91, 96, 99, 128–131), as well as in the regulation of skeletal growth (98, 132). The mechanism(s) underlying the described *ERα-VDR* interaction effect on bone mass is not known, and other studies have failed to confirm it (92–94, 97, 104, 108, 133). Other investigators have reported interactions with a polyglutamine tract polymorphism at an *ER* cotranscriptional activator gene (134), with a VNTR polymorphism at the aromatase gene (135), with the *Sp1* binding site polymorphism in the *COL1A1* gene (102, 136), and with a Gln223Arg polymorphism of the leptin receptor gene (110).

### Gene-environment interactions

Few specific gene-environment interactions have been described for *ER* gene polymorphisms. An association between the *ERα* exon 8 single nucleotide polymorphism and the Osteoporosis Self-Assessment Tool for Asians, or OSTA index (derived from age and weight), in postmenopausal Asian women was recently proposed (119). Persons with the high-risk genotype were at greater risk of developing osteoporosis, especially with advancing age or decreasing body weight. In a recent 4-year, controlled, randomized exercise intervention trial in middle-aged Finnish men, an interaction between the *ERα PP* or *Pp Pvu*II genotype and increased BMD gain during intervention was observed, while there was no BMD change in subjects with the *pp* genotype (107). Note that there appears to be a gene-environment interaction effect between dietary calcium and *VDR* genotype, which potentially may prove relevant for the observed *ERα-VDR* gene-gene interaction (115). Given the limited influence of each single polymorphism on the total variation of bone-related traits, all of these gene-environment interactions are extremely difficult to demonstrate, and very large samples are needed.

### Associations with different disorders

Interestingly, *ER* polymorphisms are associated with traits and conditions that are directly or indirectly related to bone metabolism. In particular, the *Xba*I polymorphism has been found to be significantly associated with upper-body obesity in middle-aged persons (137) and with later age of menarche in girls (138). A similar association with ovulatory dysfunction has been proposed for the G1730A polymorphism in exon 8 of the *ERβ* gene (139), while the *ERα Pvu*II RFLP correlated with the onset of natural or surgical menopause in women from the Rotterdam Study cohort (140). Other observations indicated an association between *ERα* intron I RFLPs and height (141) or body mass index (142). In a study on postmenopausal women from Finland, risk of falls (an

**TABLE 2. Findings from pharmacogenomic studies of hormonal replacement therapy and polymorphisms in the human estrogen receptor α gene**

| Study (reference no.) | Polymorphism | No. of subjects | Duration (years) of HRT* use | Genotype effect |
|---|---|---|---|---|
| Han et al. (76) | *Pvu*II, *Xba*I | 248 | 1 | No differences in lumbar spine BMD* change or biochemical markers |
| Deng et al. (128) | *Pvu*II, *Xba*I | 108 | 3.5 | Differences in lumbar spine BMD and distal radius BMD change |
| Kurabayashi et al. (133) | *Pvu*II, *Xba*I | 82 | 1 | No differences in lumbar spine BMD change |
| Salmén et al. (103) | *Pvu*II | 322 | 5 | No differences in lumbar spine BMD or femoral neck BMD change |
| Ongphiphadhanakul et al. (146) | *Pvu*II | 124 | 1 | Differences in lumbar spine BMD but not in femoral neck BMD change |
| Giguere et al. (129) | *Pvu*II | 425 | —† | Differences in heel quantitative ultrasound change in women with >5 years of HRT use |
| Salmén et al. (147) | *Pvu*II | 331 | 5 | Differences in vertebral fracture incidence |
| Bagger et al. (104) | *Pvu*II, *Xba*I | 116 | 2 | No differences in bone turnover markers |
| Ongphiphadhanakul et al. (52) | Exon 1, T262C | 96 | 2 | Differences in femoral neck BMD change |
| Kobayashi et al. (89) | *Pvu*II, *Xba*I | 58 | 1 | Differences in lumbar spine BMD change |
| Kurabayashi et al. (91) | *Pvu*II, *Xba*I | 81 | 3 | Differences in lumbar spine BMD change |

* HRT, hormone replacement therapy; BMD, bone mineral density.
† Cross-sectional study.

important factor associated with fracture risk independently of BMD) was higher in women with the *PP* genotype than in those with the *Pp* (relative risk = 5.26) or *pp* (relative risk = 3.84) genotype (143). Moreover, both the *Pvu*II and *Xba*I RFLPs appeared to influence circulating levels of androstenedione (the highest levels of hormones being found in women with the *pp* or *xx* genotype) (144), while the *ER*β gene CA repeat polymorphism was associated with variations in levels of androgen and sex hormone-binding globulin in premenopausal women (122).

## LABORATORY TESTS

Screening for intron I single nucleotide polymorphisms in the *ER*α gene is commonly carried out by means of polymerase chain reaction (PCR) amplification followed by RFLP analysis; *Pvu*II is used for T397C and *Xba*I is used for C351G. Recently, van Meurs et al. (51) proposed a direct molecular haplotyping method that consists of simultaneous RFLP digestion with both restriction sites, leading to the identification of four common haplotypes (1 = *px*, 2 = *PX*, 3 = *Px*, and 4 = *pX*). Molecular methods for determining polymorphic TA repeats upstream from the human *ER*α gene were first described by Sano et al. (75). Briefly, PCR is utilized to amplify the TA dinucleotide repeat using labeled [$\alpha^{32}$P]-deoxycytidine triphosphate. Amplified products are then separated and analyzed on denaturing polyacrylamide sequencing gel. The number of TA repeats can be determined by comparing gel bands with a series of TA size standards. More recently, fluorescein-labeled primers have been used and the number of TA repeats from PCR products has been determined automatically (51, 118). Other single nucleotide polymorphisms in the *ER*α gene have been analyzed by either direct sequencing or RFLP analysis (52–54, 119, 120).

For determination of *ER*β CA VNTR, amplified PCR fragments can be analyzed by denaturing polyacrylamide gel electrophoresis and subjected to autoradiography. The number of CA repeats can be obtained by comparison with previously determined CA size standards. Improvements of this technique have recently been developed utilizing fully automated systems for the electrophoretic separation of the PCR products (123) and fluorescent labeling of DNA fragments (122). Single nucleotide polymorphisms have been analyzed by either direct sequencing or RFLP analysis. In particular, A105G, G1082A, T1100G, and A1730G single nucleotide polymorphisms can be easily detected by RFLP analysis with the *Fok*I, *Rsa*I, *Van91*I, and *Alu*I restriction endonucleases, respectively (57, 126, 127, 139).

## POPULATION TESTING

On the basis of the evidence summarized here, testing for polymorphisms of the *ER*α and *ER*β genes in the general population as part of a population screening program is not presently warranted. The association between these polymorphisms and osteoporotic risk is intriguing but limited to some studies. Additional large-scale longitudinal studies are needed to confirm the reported associations. Moreover, the molecular mechanism underlying the skeletal effect of these polymorphisms remains to be determined.

## OTHER POTENTIAL PUBLIC HEALTH APPLICATIONS

A promising and interesting field in studies of *ER* gene polymorphisms comes from their possible pharmacogenomic implications in determining the response to hormone replacement therapy. The positive influence of postmenopausal hormone replacement therapy on bone mass is well established, and its antifracture effect is widely accepted. However, it seems that there are some women who do not respond to hormone replacement therapy (145), and a possible explanation for their less favorable responsiveness to estrogen may be related to *ER* genotypes. This issue has been investigated in a few studies performed in different

ethnic populations, with conflicting results (52, 76, 89, 91, 103, 104, 128, 129, 133, 146, 147). An updated summary of the findings of these pharmacogenomic hormone replacement therapy studies is given in table 2. Moreover, there is now consistent evidence that the *Pvu*II polymorphism in the *ERα* gene influences individual response to hormone replacement therapy with regard to cholesterol levels and other cardiovascular markers (38, 148, 149).

A further application of genetic studies on *ER* gene polymorphisms relates to the possibility of a modulation of the activity of selective ER modulators in different target tissues. In particular, raloxifene represents a potent compound for the prevention and treatment of osteoporosis in postmenopausal women (150) and possibly in men (151). Raloxifene has been shown to bind both ERα and ERβ, and it exhibits targeted antiestrogenic activity in the breast and uterus while acting as an agonist in bone and liver (152). At present, the possibility of a modulation of raloxifene's effects on bone according to *ER* genotype has not yet been investigated. However, a recent study of Japanese women treated with tamoxifen (a selective ER modulator indicated for treatment of breast cancer with adjuvant effects on bone) showed an increased gain in BMD and increased suppression of bone turnover in women with the 21 CA repeat allele in intron 5 of the *ERβ* gene with respect to noncarriers, suggesting that this polymorphism might be useful in the prediction of bone response to selective ER modulators (153).

## CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Osteoporosis affects hundreds of millions of patients throughout the world and has a great impact on both individuals and society as a whole. Its pathophysiologic basis includes genetic predisposition and subtle alterations in systemic and local hormone levels, coupled with environmental influences. Identification of the genetic pathways involved will certainly be difficult and represents a great challenge. In the past few years, several loci and genes, including *ERα* and *ERβ*, have been found to be associated with BMD and osteoporotic fractures (115, 154–156). However, the majority of these findings remain inconclusive. Reflecting the complicated inheritance patterns of osteoporosis as a complex disease, these inconsistent findings call for new approaches and strategies that have both sensitivity and robustness to accommodate confounding effects from various sources, such as genetic heterogeneity, population admixture, and gene-environment and gene-gene interactions. Definitions of disease (i.e., analysis of a single trait such as BMD, bone size, or bone quality) and pharmacogenomic interactions in human and animal models will be additional critical targets for future research.

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## APPENDIX

### Internet Sites Pertaining to Osteoporosis and Genetics

#### Information on osteoporosis

International Osteoporosis Foundation:
http://www.osteofound.org/
American Association of Orthopedic Surgeons, Osteoporosis Section:
http://orthoinfo.aaos.org/
American Society for Bone and Mineral Research:
http://www.asbmr.org/
Asian Pacific Osteoporosis Foundation:
http://www.apof.org/
The Bone and Joint Decade: Joint Motion 2000–2010:
http://www.bonejointdecade.org/
Bone and Tooth Society (United Kingdom):
http://www.batsoc.org.uk/
BoneKEy—International Bone and Mineral Society:
http://www.bonekey-ibms.org/
European Calcified Tissue Society:
http://www.ectsoc.org/
Foundation for Osteoporosis Research and Education:
http://www.fore.org/

International Bone and Mineral Society:
http://www.ibmsonline.org/
International Society for Clinical Densitometry:
http://www.iscd.org/
Osteoporosis and Related Bone Diseases National Resource Center, National Institutes of Health:
http://www.osteo.org/
National Osteoporosis Foundation (United States):
http://www.nof.org/
National Osteoporosis Society (United Kingdom):
http://www.nos.org.uk/
Women's Health Matters—Osteoporosis Health Centre, Sunnybrook and Women's College Health Sciences Centre (Canada):
http://www.womenshealthmatters.ca/centres/osteo/index.html
Società Italiana dell'Osteoporosi, del Metabolismo Minerale, e delle Malattie dello Scheletro (Italy):
http://www.siommms.it/home.htm

**Information on genetics**

Human Genome Epidemiology Network (HuGE Net):
http://www.cdc.gov/genomics/hugenet/default.htm
Public Health Genetics Unit, University of Cambridge (United Kingdom):
http://www.phgu.org.uk/index.php
Online Mendelian Inheritance in Man (OMIM):
http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html
GenAtlas:
http://www.dsi.univ-paris5.fr/genatlas
GeneCards:
http://www.cgal.icnet.uk/genecards
National Center for Biotechnology Information:
http://www.ncbi.nlm.nih.gov/
Human Genome Mapping Project—Medical Research Council (United Kingdom) (includes links to other sites via the Genome Web):
http://www.hgmp.mrc.ac.uk/